

REMARKS

Upon entry of this amendment, claims 1-7, 9-19 and 21-22 are pending in the instant application. Claims 8 and 20 were previously cancelled, and Applicants again reserve the right to prosecute that subject matter, as well as the originally presented claims, in continuing applications. Claims 1, 15, and 17-18 have been amended herein, and claims 21-22 have been added herein. Support for the claim amendments presented herein is found throughout the specification and in the claims as originally filed. For example, support for the communication modules recited by amended claims 1, 15 and 17 is found at least at page 22, lines 20-23; and at page 33, lines 11-19. Support for the process of identifying generic reporter bridging domains recited by amended claim 17 and new claim 21 is found at least at page 22, lines 20-23; in Example 1 at page 25, lines 5-25; and in Example 3 at page 33, lines 11-19. Support for the methods of identifying functional bridging domains, as recited by new claim 22, is found at least at page 6, lines 4-15; at page 8, lines 8-17; and in Example 1 at page 25, lines 5-25. Accordingly, no new matter has been added by these amendments.

I. Claim rejections under 35 U.S.C. §102

The rejection of claims 1-7, 9, 10, 12-16 and 19 under 35 U.S.C. § 102(a) as being anticipated by Araki *et al.*, *Nucleic Acids Research*, vol. 26(14): pp. 3379-3384 (1998) (“Araki”) has been maintained by the Examiner “for the same reasons as set forth in the Official Action mailed 1/2/03”. (Office Action, p. 2).

Similarly, the rejection of claims 1-7, 9, 10, 12-16 and 19 under 35 U.S.C. § 102(b) as being anticipated by Tang *et al.*, *Chemistry and Biology*, vol. 4(6): pp. 453-59 (1997) (“Tang”) has also been maintained by the Examiner “for the same reasons as set forth in the Official Action mailed 1/2/03”. (Office Action, page 2).

The Examiner has indicated Applicants’ arguments in response to these rejections, filed in the May 2, 2003 Response and Amendment, have not been found persuasive. According to the Examiner, “the specification as filed does not provide a specific definition of what a randomized domain is in the context of the claim as now amended.” The Examiner has asserted that the “randomized bridging domain” limitation could be interpreted as “a randomized bridging

sequence which has been found to have the function screened for,” which, in the context of the specification, could be compared to a product by process limitation. (Office Action, page 3).

Applicants note that the claims 1 and 15 (and their respective dependent claims, including claims 2-7, 9, 10, 12-14, 16 and 19) have been amended herein to delete all references to a randomized bridging domain. As amended herein, independent claim 1 is directed to a purified, functional, tripartite polynucleotide that includes three functional domains: (i) an actuator domain, (ii) a receptor domain, and (iii) a bridging domain that includes a communication module, wherein the communication module is a generic reporter of the occupation state of the receptor domain, and wherein interaction of the receptor domain with a signaling agent triggers a conformational change in the bridging domain, which, in turn, modulates the activity of the actuator domain.

Independent claim 15, as amended herein, recites a process for preparing polynucleotides that are responsive to the presence or absence of a signaling agent by linking together three functional domains, a polynucleotide actuator domain, a receptor domain, and a bridging domain that includes a communication module that is a generic reporter of an occupation state of the receptor domain, such that interaction of the signaling agent with the receptor domain triggers a conformational change in the bridging domain which modulates the activity of the actuator domain.

Thus, claims 1 and 15 (and their respective dependent claims) are directed to polynucleotides that include a functional bridging domain having a communication module that acts as a generic reporter of the occupation status of the receptor domain (*i.e.*, whether the signaling agent is present (*e.g.*, bound) or absent (*e.g.*, unbound)), as well as methods of producing and screening for such polynucleotides. In other words, the functionality of the claimed bridging domain is not dependent on being coupled to a particular receptor domain, or the presence of a specific receptor domain/signaling agent interaction. Rather, this type of bridging domain – the generic reporter – modulates the activity of the actuator domain when coupled to a variety of aptamers (*i.e.*, receptor domains). Thus, the claimed generic reporter bridging domains function “regardless of the particular ligand specificity.” (*See* specification, page 33, lines 11-19).

Araki and Tang, however, fail to describe or suggest multidomain polynucleotides having a bridging domain that is a generic reporter on the occupation state of the receptor domain.

Araki

The allosteric ribozymes disclosed by Araki contain a known aptamer (*i.e.*, the FMN binding loop), a known ribozyme (*i.e.*, the hammerhead ribozyme), and a rationally designed linking region (*i.e.*, one of the six constructs disclosed in Figure 1b). Araki explicitly teaches that the six linking region constructs shown in Figure 1 were specifically designed to contain the U-A base pair of the FMN-binding loop and a series of G-C base pairs, as “the FMN aptamer was previously found to have conformational characteristics involving formation of a short helix of A-U/G-C pairs on ligand binding.” (See p. 3380, col. 2, “Results and Discussion,” first paragraph). In other words, the linking regions of the Araki allosteric ribozymes were designed to mimic the short helix of A-U/G-C pairs that forms when a ligand binds to the FMN aptamer.

Thus, the linking regions of Araki were *rationally designed* using previous knowledge about the structure and function of the FMN aptamer in order to optimize the linking function with regard to *that specific aptamer*. There is no teaching or suggestion in the Araki reference that the bridging domains shown in Figure 1 can be used with *any* aptamer other than the FMN aptamer, let alone that the Araki linking regions would retain the same, or possess better, function (*i.e.*, the ability to modulate the activity of the hammerhead ribozyme domain), when coupled to a variety of aptamer domains. Araki, therefore, fails to disclose or suggest every element of the claimed polynucleotides and methods. Accordingly, claims 1-7, 9, 10, 12-16 and 19 are novel over this reference, and Applicants request that the Examiner withdraw this rejection.

Tang

Like the Araki allosteric ribozymes, the Tang ribozyme constructs also contain a known aptamer (*i.e.*, the ATP-binding aptamer), a known ribozyme (*i.e.*, the hammerhead ribozyme), and a rationally designed linking region (*i.e.*, one of the seven linking region constructs (H1-H7) shown in Table 1). Tang teaches that these seven linking region constructs either contained the known linking regions of the ATP-aptamer or the hammerhead ribozyme, or alternatively, the linking regions were *specifically derived* from these known linking regions. For example, the constructs labeled H1 and H2 contained the known stem II region of the hammerhead ribozyme,

while constructs H3 and H4 included the known stem II region of the ATP-aptamer. Construct H5 was specifically designed to include a 3 base-pair extension within the known stem II region of the ATP-aptamer, and constructs H6 and H7 were specifically designed to replace four base pairs in the stem II region of the polynucleotide with “less stable G-U mismatches.” (See p. 456, col. 2, second paragraph). In fact, Tang acknowledges “we intended to exploit the fact that the G-C pair that begins stem II within the aptamer domain is not paired in the absence of ATP, but will form a stable pair when ATP is complexed.” (*Id.*) In other words, the linking regions of Tang include either previously known sequences, or sequences that have been rationally designed using previous knowledge about the structure and function of the ATP aptamer. Thus, the linking regions described by Tang were *rationally designed* in order to optimize the linking function with regard to *one specific* aptamer, the ATP aptamer.

Again, Applicants contend that there is no teaching or suggestion in the Tang reference that constructs H1-H7 can be used with *any other* aptamers, let alone that the Tang linking regions would retain the same or better functionality (*i.e.*, the ability to modulate the activity of the hammerhead ribozyme domain), when coupled to a variety of aptamer domains. Accordingly, Tang also fails to describe or suggest every element of the claimed polynucleotides and methods. Thus, claims 1-7, 9, 10, 12-16 and 19 are novel over this reference, and this rejection should be withdrawn.

II. Claim rejections under 35 U.S.C. §103(a)

The rejection of claims 11, 17 and 18 under 35 U.S.C. § 103(a) as being unpatentable over Araki, Tang and Breaker *et al.*, *Chem. Rev.*, vol. 97: pp. 371-390 (1997) (“Breaker”) has been maintained by the Examiner “for the same reasons as set forth in the Official Action mailed 1/2/03”. (Office Action, p. 2). The Examiner has indicated that Applicant is “putting the cart before the horse and arguing Breaker in view of Tang.” According to the Examiner, “since Tang has taught the compounds of the invention ... the method of Breaker et al could be used to screen for improvements.”

Applicants traverse. Claim 11 depends indirectly from claim 1 and, therefore, necessarily contains all of the limitations recited by claim 1. As described above, claim 1 has been amended herein to recite a purified, functional, tripartite polynucleotide that includes three functional

domains: (i) an actuator domain, (ii) a receptor domain, and (iii) a bridging domain that includes a communication module, wherein the communication module is a generic reporter of the occupation state of the receptor domain, and wherein interaction of the receptor domain with a signaling agent triggers a conformational change in the bridging domain, which, in turn, modulates the activity of the actuator domain.

Amended claim 17 is directed to a process for identifying generic bridging domains that modulate the activity of two or more different actuator domains by (i) preparing a pool of polynucleotides, wherein each polynucleotide in the pool includes a first actuator domain having a pre-existing actuator domain nucleotide sequence and a receptor domain having a pre-existing receptor nucleotide sequence linked by a randomized bridging domain comprising a random nucleotide sequence, wherein the receptor domain is responsive to a first signaling agent; (ii) incubating a sample containing the first signaling agent with the polynucleotide pool and identifying which polynucleotides in said pool are responsive to the presence of the first signaling agent; (iii) determining the nucleotide sequence of the randomized bridging domain in each responsive polynucleotide identified in order to identify functional bridging domains that modulate the activity of the first actuator domain; and (iv) determining whether each functional bridging domain modulates the activity of at least a second actuator domain in the presence of at least one signaling agent that is different from the first signaling agent, thereby identifying generic bridging domains that modulate the activity of two or more different actuator domains.

Thus, claims 1 and 17 (and their respective dependent claims, including claims 11 and 18) are directed to polynucleotides that include a functional bridging domain having a communication module that acts a generic reporter of the occupation status of the receptor domain (*i.e.*, whether the signaling agent is present (*e.g.*, bound) or absent (*e.g.*, unbound)), as well as methods of identifying such polynucleotides. In these claimed polynucleotides and methods, the functionality of the claimed bridging domain is not dependent on being coupled to a particular receptor domain, or the presence of a specific receptor domain/signaling agent interaction. Rather, this type of bridging domain – the generic reporter – modulates the activity of the actuator domain when coupled to a variety of aptamers (*i.e.*, receptor domains). As described in the instant specification, *e.g.*, at page 33, lines 11-19, the claimed generic reporter bridging domains function “regardless of the particular ligand specificity.”

However, as described above, Araki and Tang each fail to describe or suggest multidomain polynucleotides having a bridging domain that is a generic reporter on the occupation state of the receptor domain. The bridging domains described by Araki and Tang were all *rationally designed* to improve the linker function with regard to *one specific* aptamer, and, moreover, there is no teaching or suggestion in these references that the disclosed linker regions can be used with *any other* aptamers or any other signaling agents. The addition of Breaker fails to remedy these deficiencies in the teachings of Araki and Tang.

In contrast to the teachings of the present specification, Breaker fails to describe or suggest polynucleotides, or methods of identifying polynucleotides, having a generic bridging domain that includes a communication module that acts as a generic reporter of the occupation state of the receptor domain. The *in vitro* screening methods taught by Breaker are used to isolate novel catalytic nucleic acids from pools of completely random RNA sequences. According to Breaker, this approach “relies on the probability that a given pool of random sequence molecules will include individuals that can perform the function of interest” (*i.e.*, catalytic activity). (Breaker, p. 372, col. 2). Thus, the *in vitro* selection methods taught by Breaker identify fully randomized ribozyme sequences.

There is no teaching or suggestion in the Breaker reference to produce multidomain polynucleotides having a bridging domain that would retain the same or possess better functionality (*i.e.*, the ability to modulate the activity of a catalytic ribozyme domain), when coupled to a variety of different aptamer domains. Similarly, Breaker also fails to disclose or suggest methods of screening for polynucleotides that contain such a bridging domain. Accordingly, Breaker fails to describe or suggest the polynucleotides and screening methods of the claimed invention.

Moreover, Applicants once again contend that the combination of Tang and Breaker fail to disclose or suggest the polynucleotides and methods recited by claims 1 and 17 (and their respective dependent claims). At page 458, col. 1, Tang suggests the possibility of refining the interplay between the aptamer and ribozyme motifs to improve ribozyme catalytic rates. Citing Breaker, Tang suggests such improvements can be made using “a combinatorial library of RNAs followed by screening *via in vitro* selection.” Tang, however, fails to disclose or suggest any

methods of refining the interplay between the aptamer and ribozyme motifs of the disclosed allosteric ribozyme constructs, other than the *in vitro* selection methods disclosed in Breaker.

As described above, the *in vitro* screening methods taught by Breaker are used to identify fully randomized ribozyme sequences. Thus, the combination of Breaker and Tang teaches an allosteric ribozyme construct having a randomized actuator domain (*i.e.*, the randomized ribozymes identified by the Breaker *in vitro* screening methods) coupled to the ATP aptamer (*i.e.*, the receptor domain) via a rationally designed linking region. Accordingly, Tang and Breaker do not teach or suggest the claimed polynucleotide constructs, which contain an actuator domain having a known actuator nucleotide sequence coupled to a receptor domain having a known receptor nucleotide sequence via a randomized bridging domain.

As the Araki, Tang and Breaker references, alone or in combination, fail to teach or suggest every element of the claimed polynucleotides and screening methods, claims 11, 17 and 18 are novel and nonobvious over these references. Applicants, therefore, request that the Examiner withdraw this rejection.

Applicants also note that new independent claim 22 recites methods for identifying functional bridging domains that modulate the activity of an actuator domain by (i) preparing a pool of polynucleotides, wherein each polynucleotide in the pool includes an actuator domain having a pre-existing actuator domain nucleotide sequence and a receptor domain having a pre-existing receptor nucleotide sequence linked by a randomized bridging domain having a random nucleotide sequence, wherein the receptor domain is responsive to a signaling agent; (ii) incubating a sample containing the signaling agent with the polynucleotide pool and identifying which polynucleotides in the pool are responsive to the presence of the signaling agent; and (iii) determining the nucleotide sequence of the randomized bridging domain in each responsive polynucleotide, thereby identifying functional bridging domains that modulate the activity of the actuator domain.

As described above, Applicants contend that neither Tang, Araki nor Breaker, alone or in combination, disclose or suggest methods of using a pool of nucleotides having an actuator domain and a receptor domain linked by a randomized bridging domain having a random nucleotide sequence to identify functional bridging domains that modulate the activity of the actuator domain. Rather, the bridging domains disclosed by Tang and Araki are rationally

designed to improve functionality with regard to a *specific* aptamer and/or ribozyme. Moreover, Breaker is directed to *in vitro* selection methods for identifying fully randomized ribozyme sequences. Therefore, Applicants contend that there is no teaching of suggestion in any of these references that would motivate one of ordinary skill in the art to create pools of polynucleotides having a known actuator domain and a known receptor domain linked via a randomized bridging domain to identify functional bridging domains that modulate the activity of the actuator. Accordingly, Applicants contend that claim 22 is also novel and nonobvious over the teachings of the prior art.

CONCLUSION

On the basis of the foregoing amendments, Applicants respectfully submit that the pending claims are in condition for allowance. If there are any questions regarding these amendments and remarks, the Examiner is encouraged to contact the undersigned at the telephone number provided below.

Respectfully submitted,



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